STUDIES ON POST- HARVEST COLD STRESS IN TOMATO

SHORT SYNOPSIS

Submitted in partial fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

to

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by

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Certificate

I here by declare that the Short Synopsis titled "Studies on Post-Harvest Cold Stress in Tomato " being submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the Department of biotechnology under School of Engineering &Technology of Manav Rachna International Institute of Research & Studies, Faridabad during the academic year 2023-2024. It is a bona fide record of original work carried out under the supervision of Dr. Abhilasha Shourie, Professor, Department of Biotechnology, SET,MRIIRS, has not been submitted in full or part to any other university or institute for degree or diploma. Further, it is certified that Short Synopsis is plagiarism free and proof has been submitted.

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Abstract

India is the second-largest tomato grower in the world and contributes around 11% to the total world's tomato production which amounts to approximately 20 million metric tons. Tomato is majorly produced in five states in India i.e. Madhya Pradesh, Andhra Pradesh, Karnataka, Telangana and Gujarat, and is further supplied to different parts of the country through the supply chain that includes long distance transportation under cold chain and long or short-term cold storage. Tomato is highly perishable with small shelf life and high post-harvest loss, due to which its market prices fluctuate from Rs.30per Kg to Rs.109 per Kg from June to end of July months Since June and July fall between the summer and monsoon harvests, there is always a price increase for tomatoes during those months and a decrease until mid-August. Post-harvest losses due to tomato handling occur at various levels such as field or farm level, market level, transportation, storage and retailer level. In India, there is a 13% post-harvest loss on tomatoes, of which 3.04% is caused during transportation and storage. Since tomato is chilling sensitive, one of the major factors contributing to this loss is exposure of tomato fruit to low temperature in the cold chain. The recommended temperature range for storage and transportation of tomato is 10-13°C at 90% relative humidity, however, often the temperature is maintained at 3-6°C in most cold storage supply chain. Storage of tomato at the temperature < 10°C cold stress is induced, while at temperature below 4°C chilling injury is induced that leads to loss of aroma, appearance of blotch, softened texture, disease susceptibility and inability to adequate ripening. As tomato is a climacteric fruit, its ripening is induced and influenced by ethylene, which also plays significant role in cold stress management. This research work aims to study the effect of post-harvest cold stress on tomato and assess the role of ethylene in alleviation of cold-stress and modulation of the ripening process of tomato fruit under cold stress.

Keywords: Tomato production, post-harvest loss, cold stress, chilling injury, ethylene modulators

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown crops, and its fruits which are priced for their organoleptic qualities and nutritional worth, are consumed globally. Around 864 thousand hectare of land is used for tomato cultivation in India, and India being the second-largest tomato grower in the world and contributes around 11% to the entire world's tomato production that is approximately 20 million metric tons. Tomato is majorly produced in five states in India i.e. Madhya Pradesh, Andhra Pradesh, Karnataka, Telangana and Gujarat, of which the highest tomato producers in India are Madhya Pradesh with production of 2805.07 thousand metric tons, Andhra Pradesh with 23.38 lakh tones, and Karnataka with a production of 2077 thousand metric tons in the year 2022–23 (NABARD 2023).

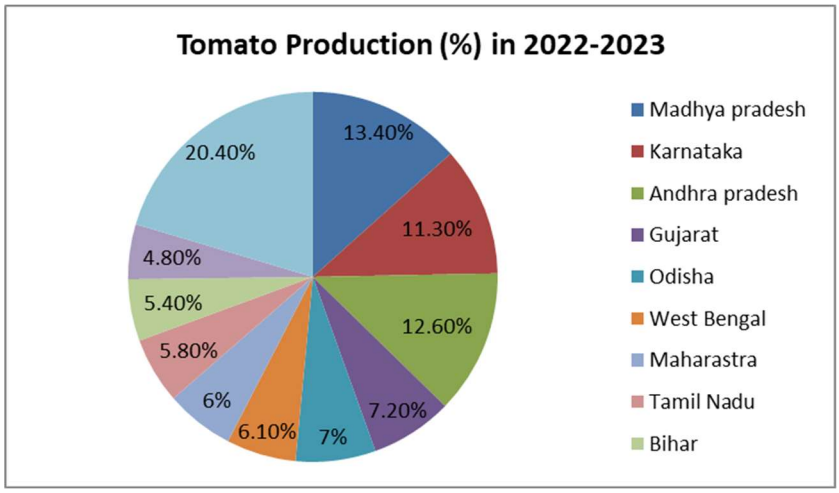

Figure-1: Major Tomato Producing States in India.

Tomato is supplied from the major tomato producing states to different parts of the country and the supply of tomato is maintained throughout the year as the harvesting seasons are different in these states, i.e. January, February, October and December in Madhya Pradesh,

January to December in Andhra Pradesh and Telangana, May to July in Karnataka, and January, February, March and December in Gujarat. The supply chain of tomato includes long distance transportation and long or short-term storage of tomato fruit. Tomatoes are transported by truck, tractor, train, and airway to distant markets. Large quantity of tomato is also stored in godowns provided by the Government under the scheme of Grahmin Bhandar Yojana or in private storages.

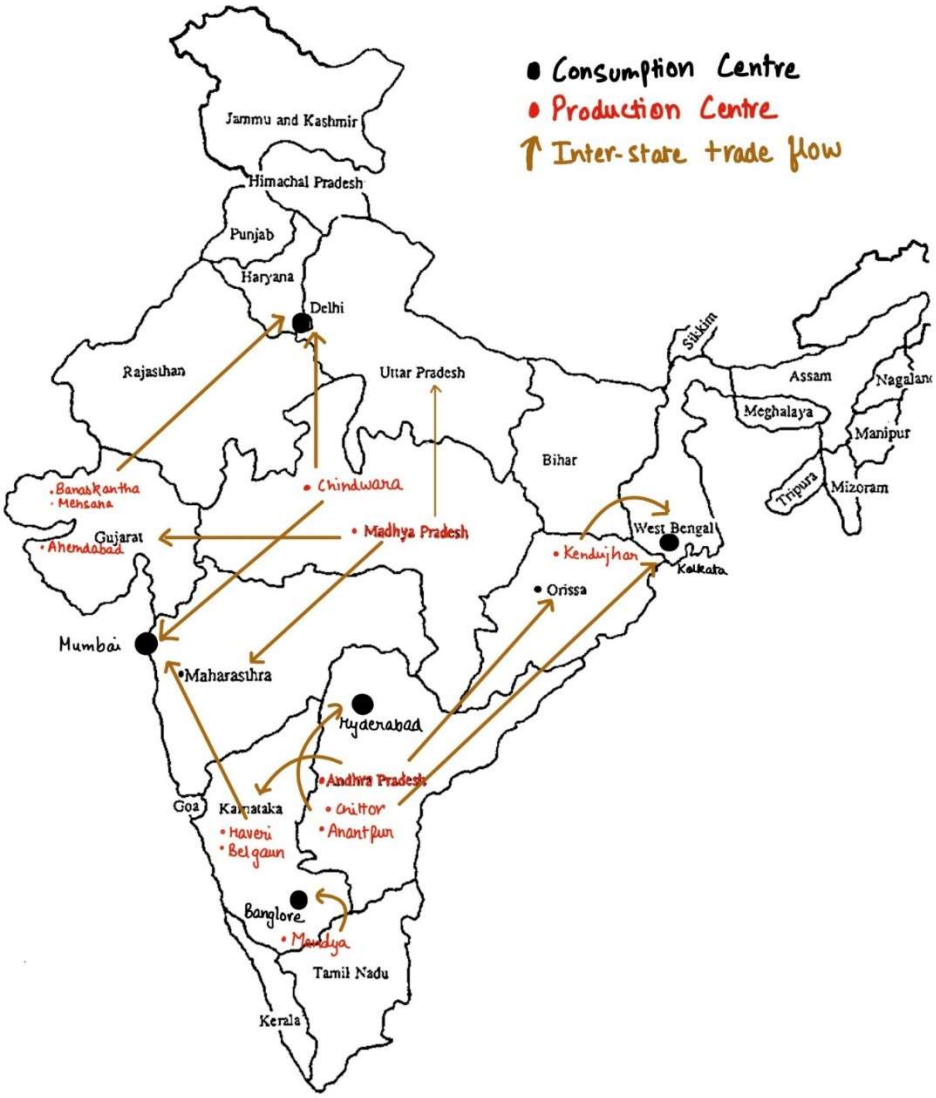

Figure-2: Inter-state Tomato Trade-flow across India

The post-harvest losses of tomato are accounted to be approximately 40% of the total produce, of which the major losses occur due to tomato handling at various levels such as field or farm level, market level, transportation, storage and retailer level. These losses include cracks, pathogen infestation, insect damage, rotting, etc. In India, there is a 13% post-harvest loss on tomatoes, of which 9.94% occurs in the field and 3.04% is caused by storage (Rai et al., 2022). The post-harvest loss is one of the major factors contributing to the irrational pricing of tomato in the retail market. According to NABARD 2023 report, selling price of tomato in India was about ₹30 per kg in June 2023, which increased to ₹109 per kg in the month of July 2023, with the inflation rate of 64.46%. Since June and July fall between the summer and monsoon harvests, there is always a price increase for tomatoes during those months and a decrease until mid-August. The fluctuating prices of tomato has been a major economic concern in the commodity market, giving good fortune to a few while posing problems for millions. On the other hand, the sharp decline in tomato prices in recent months has put farmers through hardship and in some cases, forced them to discard their tomato harvest in the fields.

Post harvest losses largely depend upon several factors such as the harvesting stage, in-transit duration, mode of transport and physical conditions maintained during transportation and storage. Tomato fruit is harvested at six stages which are Green Mature Stage, Breaker Stage, Turning Stage, Pink Stage, Light Red Stage and Red stage. Tomato fruit at Green Mature Stage is bigger in size, rigid, green and has little taste. Tomato is harvested at this stage to be sent to distant places through transportation. Breaker, Turner and Pink stages are also transported and/ or stored for some duration. Red tomatoes are readily perishable and therefore are preferably sold in the local market shortly after harvest (Choi et al., 2023).

Since tomato is a climacteric fruit and ripens fast it is best stored and transported at low temperatures depending on the stage of fruit development, in order to delay ripening and extend the shelf life. The perishable nature of tomato thus necessitates its storage and transportation through cold chain. The optimum temperature recommended for storage of tomato is 10-13 °C with 90% relative humidity, at which the majority of fruit remains fresh for two to three weeks. Unripe tomatoes can be stored in cold storage at around 10 °C, with RH or relative humidity upto 90 % for a maximum of four weeks, while 7°C with 90% relative humidity are appropriate conditions for ripe fruits to be stored for a week (Ayomide et al., 2019).

Tomato is cold/ chilling sensitive fruit and its exposure to temperature < 10^{o}C causes cold stress and temperature < 4oC causes induction of chilling injury symptoms in the fruit. Fruits stored at low temperatures exhibit chilling induced surface pitting, poor fruit color development and rapid deterioration after rewarming. Cold stress in tomato causes several physiological or cellular disruptions. Storage of tomato at freezing temperature leads to chilling injury due to development of ice crystals which may eventually destroy the fruit's meshy texture and cell integrity (Biswas et al., 2016). Fruit must preserve cell behavior and function during LT stress, especially the membrane integrity and the structure of the biologically active protein, in order to survive in unfavorable conditions. Ice crystals initially develop in the extracellular region of the cell eventually in the cytoplasm. Outward flow of water from cells lowers the water potential in the region of apoplast which results into dehydration stress followed by freezing stress. Electrolyte leakage is also observed to be increased along with the phase shifts in membrane lipids during freezing stress (Ritonga et al.,2020).

Primary and secondary symptoms that are typical indicators of cold stress and chilling injury are lesser development of aroma, appearance of blotch like symptoms, softening and disease susceptibility, and inability to adequate ripening. Cold stress and chilling injury in tomato fruits is assessed by visual characterization and physiological tests for membrane damage and reactive oxygen species metabolism. The cold stress response involves cold-induced gene expression and induction of stress signaling pathway mediated by ethylene. There is direct influence of cold stress on the tomato fruit quality as it can cause chlorophyll degradation, and alterations in carotenoid and lycopene accumulation (Biswas et al., 2016).

Tomato is a climacteric fruit, and the entire process of fruit ripening is controlled and modulated by ethylene production. Thus, two essential mechanisms for fruit ripening are ethylene production and signal transmission (Houben et al.,2019). Ethylene modulators are commonly used to control ripening and extend shelf life of tomato during transportation and storage. Chemicals such as 2-aminoethoxyvinyl glycine (AVG) and amino-oxy-acetic acid (AOA) are the inhibitors of ethylene biosynthesis, whereas 1-methylcyclopropene (1-MCP) is an inhibitor of ethylene activity. 1-MCP is a synthetic compound with a structure very similar to ethylene. It prevents the fruit from sensing ethylene, which delays post-harvest ripening. Although this can increase fruit hardness, preserve fruit nutrients and quality, and increase the longevity and shelf life of tomato, it can also have unfavorable consequences including exacerbating physiological stress while in storage (Nascimento et al., 2018).

Since ethylene plays role in both the processes that is fruit ripening and cold stress signaling, it is imperative to understand the inter-linking features of ethylene mediated mechanisms.

There are no studies that report the effect of cold-stress on different ripening stages of tomato and the effect of ethylene modulators in various stages of tomato under cold stress has also not been elucidated. This study will be undertaken to investigate the effect of low temperature exposure on tomato fruits and evaluate the post-harvest cold stress and chilling injury. The role of ethylene in alleviation of cold-stress and control of the ripening process of tomato fruit under cold stress will be studied. The prospects of using ethylene modulators for alleviation of cold stress in tomato and enhancement of shelf life will also be explored.

2. Review of Literature

Globally, post-harvest losses of tomatoes reach upto 42%, which include both the quantitative and qualitative losses. There is minimal improvement in the quality of tomato varieties used for commercial purposes, despite the fact that agricultural research is increasingly focusing upon the nutritional aspects of the produce. Several studies show that pre-harvest production practices had an influence on the postharvest quality status of tomatoes. Fertilizer use, trimming, maturity stage, cultivar selection, and irrigation are a few of these elements. It was crucial to use the best postharvest handling techniques and standardize the variables to preserve the nutrition and quality of fruits (Datta et al.,2024).

The production and quality of fruit are best understood in relation to the function that ethylene plays important role in the process. Tomatoes are climacteric fruits, and ethylene burst is necessary for normal fruit ripening. Biosynthesis of phytohormone ethylene in tomato in two phases- the auto-inhibitory phase and the catalytic phase. In auto-inhibitory phase occurs at the start of ripening, when fruit contains basal levels of the phytohormone and a decreased ethylene

response. The catalytic phase follows the auto-inhibitory phase during which the synthesis of ethylene increases quickly as a result of extreme sensitivity to the phytohormone. Ethylene biosynthesis pathway involves reaction of S-adenosylmethionine (SAM) with ACC-synthase (ACS) to form 1-aminocyclopropane-1-carboxylic acid (ACC) and in the subsequent step ACC is converted into ethylene by ACC-oxidase (ACO). The tomato is a useful model system to investigate the role of ethylene in ripening because of its short life cycle, well-characterized developmental mutations, accessibility of genetic manipulation, and economic significance as a crop species. Fruit turns red as it ripens because carotenoids build up, chlorophyll is broken down, and transition of chloroplasts into chromoplasts happens. Temperature affects the biosynthesis of ethylene in a complicated fashion that changes according to plant species, tissue type, maturity period, and temperature (Nguyen et al., 2024).

Peel color change and ethylene production were both suppressed by storage at 5°C; however, 1-MCP treatments during rewarming at 20°C only prevented peel color change, not ethylene production. To learn more about how fruits react to low temperatures, researchers looked at how the 1-methylcyclopropene (1-MCP) affected fruit ripening, being a powerful inhibitor of ethylene mediated signaling, the incidence of chilling injury and gene expression in mature green tomatoes stored at low temperatures. Additionally, independent of 1-MCP treatment, fruits that had been kept at 5°C for 14 days began to exhibit CI signs during the re-warming phase. An extensive transcriptome adjustment is brought about by cold stress, as evidenced by the fact that significantly more genes showed differential expression at 5°C (8,406) than at 20°C (4,814) after further RNA-Seq analysis (Mitalo et al., 2023).

The effect of modified atmosphere packaging (MAP) on tomato quality was investigated in which a MAP-filled Xtend® bag was used to transport tomatoes that were collected at two distinct stages (breaker and pink stage). Fruits kept in the air were used as the standard. All samples were held for 21 days at 5 °C and 90% RH. With intervals of seven days, researchers looked at changes in weight, hardness, surface color, lycopene, ascorbic acid, total phenol, total antioxidant activity, and chilling damage. When compared to the control fruit, the weight loss, hardness, and metabolic alterations caused by MAP were less pronounced in the breaker or pink fruits at the end of storage. In addition, tomatoes kept in MAP suffer from less chilling damage than controls at the breaker maturity stage. When compared to fruits that weren't wrapped, packing also delayed the start of the chilling harm. Fruits preserved in MAP had greater all-around properties than fruits kept in air. According to the overall research, MAP can be a useful technique for increasing the phyto-chemical content, postponing senescence, and preventing chilling injury in tomatoes during low temperature storage when they are at the breaker or pink maturity stages (Sabir et al., 2020).

The effect of alternate low and high temperature change stimuli on anti-oxidative responses in ripe tomatoes of the cv. "Sanibel" was studied. Fruit that was fully ripe was given variable heating and chilling treatments, or left untreated as the control. The samples were collected either immediately or periodically. Following treatments, heating significantly boosted the activity of lipoxygenase (LOX) that catalyses the oxidation of lipids, while cooling significantly raised the amount of malondialdehyde (MDA). On day 4 following treatment, chilling immediately elevated MDA and ascorbate levels and produced a notable rise in H2O2. On the fourth post-treatment day, cooled fruit had lower CAT and POD activity than the

control. These findings suggest that chilling treatment can cause oxidative stress even if the fruit surface is not visibly damaged. In order to potentially shield fruit from environmental damage, heat treatment improved the antioxidant enzyme system (Meitha et al., 2020).

Fruits exposed to non-freezing but low-temperatures for an extended length of time, however, run the risk of developing chilling injuries, which are physiological conditions brought on by cold. According to the fruits' susceptibility to low temperatures, chilling injuries constitute an economically significant concern that may manifest themselves in an unforeseen manner. This article focused upon the key elements of the signaling and reactions to cold stress that occur in fruits whilst storage and the subsequent time of warming, which frequently leads to the additional induction of chilling damage symptoms. Recent research has helped to illuminate several early regulatory processes that may help determine the fruit's vulnerability to cold stress. For the purpose of preventing losses throughout the supply chain, it is crucial to improve the conditions of cold storage and make effective use of resources (Cainelli et al.,2019).

Postharvest problems of tomato crop have received less attention as compared to its production, which resulted into lesser profits and sustainability challenges for tomato growers, particularly in underdeveloped nations where tomato is adequately produced but large produce is lost after harvest. Many postharvest handling procedures and treatments have profound impact on the fruit's postharvest quality and shelf life of tomato. Harvesting, pre-cooling, washing, sanitising, grading, sorting, packing, storing, and transportation practices all play a significant part in preserving quality and prolonging shelf life. Application of 1-methylcyclopropene (1-MCP), calcium chloride ($CaCl_2$), modified atmosphere packing

(MAP), refrigeration, and heat treatment are examples of acceptable postharvest treatments. The quality of the harvested fruit may be preserved and its shelf life could be increased by employing the proper post-harvest handling practices (Arah et al., 2016).

3. Description of the study:

Tomato is a climacteric fruit and is highly perishable. The ripening process of tomato is regulated by ethylene; therefore, ethylene modulators are commonly used to control its ripening and increase the shelf life. Depending on the stage of fruit development, it is recommended to store and transport them under controlled conditions of temperatures and humidity to delay ripening and prolong their shelf life. Adequate conditions for tomato storage are temperatures between 10 and 13°C with 90% relative humidity. During transportation and storage, often tomato fruits are kept at low temperature (<10ºC) for short or long durations. Since tomato fruit is chilling sensitive, the exposure to low temperature is likely to induce cold stress and chilling injury causing huge post-harvest losses. The cold stress and chilling injury differently affect the various stages of tomato fruit that is green mature, breaker, turner, pink, light red and red stage. When tomatoes are stored at or below freezing temperatures, ice crystals begin to form and may eventually cause the fruit's meshy texture and cell integrity to be destroyed (Biswas et al., 2016). Studies detailing how different tomato fruit ripening stages are affected by storage temperatures were not found. It has also not been investigated how ethylene modulators affect tomatoes during cold storage conditions. This research work will focus to investigate the post-harvest cold stress on biophysical and biochemical characteristics of various ripening stages of tomato. The impact of exogenous application of ethylene modulators on tomato under cold stress will also be evaluated.

4. Objectives of the Study-

1. To study the effect of cold stress on biophysical and biochemical parameters of various post-harvest stages of tomato.
2. To assess the effect of exogenous application of ethylene modulators on tomato under cold stress.
3. To investigate the strategies for alleviation of cold stress and enhance shelf life, while maintaining nutritional attributes of tomato.

5. Methodology

5.1 Fruit sampling and experimental setup:

Tomatoes (*Solanum lycopersicum* L.) samples will be collected at six different ripening stages- green mature, breaker, turner, pink, light red and red stage. The fruits will be sorted visually for uniformity of size and spotless appearance, and will be subjected to surface sterilization protocol. The fruit samples will be exposed to different low temperature treatments for various time durations to assess the impact of cold stress. The samples will be withdrawn from cold stress and effect of rewarming will be assessed. All the samples will also be investigated under the same conditions to observe the effect of ethylene modulators. For biochemical analyses, tomato fruit samples will be homogenized in buffer or extracted in appropriate solvent and will be frozen at -20°C in vials until further use. The samples/ aliquots will be thawed at 40°C with agitation right before analysis. Each experimental set will consist of at least 6 replicates and will be compared with control (Diretto et al.,2020).

5.2 CO2 and ethylene gas analysis:

Fruits will be put into 500 mL jars and attached to an air stream with controlled flow rate and humidified with 95% relative humidity. Periodically 1 mL of gas will be withdrawn from the containers and introduced to CO2 analyzer to determine the concentration of carbon dioxide. The respiration rates will be determined by calculating the difference between the carbon dioxide concentrations at the input and output. The amount of ethylene in the gas will be determined by introducing 3 mL of gas samples withdrawn from the samples into a gas chromatograph via a sampling port on the sealed containers. Calibration will be done using 1.022 ppm of ethylene as the standard (Albornoz et al., 2019).

5.3 Chilling injury index (CII):

Fruits samples will be evaluated visually for CII using a five-point rating system based on three factors: surface pitting, uneven ripening and deterioration. The score ranging from 0 to 4 will be used to determine the degree of CI.

CII will be computed as-

CII= (Injury level of surface pitting + Injury level of uneven ripening + Injury level of deterioration or decay)/3

The CII will be determined for all the samples separately at different time periods and values for each set will be averaged (Albornoz et al., 2019).

5.4 Carotenoid Analysis

Carotenoid analysis will be done using gas chromatography and mass spectroscopy system (GC-MS). Carotenoids will be extracted from tomato fruit samples through solvent

fractionation using organic solvents. Samples will be injected (injector temperature 200ºC) into agas chromatograph connected to a mass selective detector, and will be left for 10 minutes to allow for the desorption of volatile organic compounds. The biochemical components will be separated in a capillary column. The mass spectral library will be used to corroborate the spectral data for identification of volatile organic compounds. Additional methods of identification will include comparing the retention periods and mass spectra with those of reliable external standards, as well as the retention indices (RIs) of a range of compounds with those documented in the literature (Tobaruela et al., 2021).

5.5 Cuticle composition analysis

The cuticular waxes and cutin present in the tomato peel will be analyzed by GC-MS. The column separation of cuticular compounds will be done in a gas chromatography column using He as the carrier gas and a flame ionization detector (FID) will be employed. The mass spectral library will be used to corroborate the spectral data for identification of analytes or the analytes will be manually analyzed using peak areas to quantify them against the internal standard (González Moreno et al., 2023).

5.6 Primary metabolite analyses

The analysis of non-polar metabolites in tomato samples will be performed by GC-MS using standard protocol as stated above. The semi-polar and polar metabolites will be analyzed using liquid chromatography coupled to a diode array detector (LC-MS/MS). The analysis of phenolphthalanoid compounds will be carried out by comparing the spectrum and chromatographic characteristics with reference spectra and standards (Diretto et. al., 2020).

6. Expected outcome

Post-harvest losses of tomato due to cold stress caused during cold chain transportation and storage are huge and could be prevented through appropriate strategy. This research work will provide insights into the mechanisms by which ethylene not only regulates ripening in tomato, but also orchestrates several events underlying cold stress signaling. Through this study, the effect of ethylene modulators on ripening and regulation of cold stress in tomato fruits exposed to low temperature will also be evaluated. The research work would lead to determination of a strategy to reduce cold stress, prevent chilling injury, increase shelf life and maintain nutritional attributes of tomato while being exposed to low temperature, through exogenous application of ethylene modulators.

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Biophysical and biochemical investigations of Post-harvest Cold stress in Tomato

by Chanchal Rawat

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Biophysical and biochemical investigations of Post-harvest Cold stress in Tomato

SHORT SYNOPSIS

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Regn. No- 21/PhD /037 /MRIIRSPHD005

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ABSTRACT:

India is the second-largest tomato grower in the world and contributes around 11% to the total world's tomato production which amounts to approximately 20 million metric tons.Tomato is majorly produced in five states in India i.e Madhya Pradesh,Andhra Pradesh,Karnataka, Telangana and Gujarat, and is further supplied to different parts of the country through the supply chain that includes long distance transportation under cold chain and long or short-term cold storage. Tomato is highly perishable with small shelf life and high post-harvest loss, due to which its market prices fluctuate from Rs.30per Kg to Rs 109 per Kg from June to end of July months Since June and July fall between the summer and monsoon harvests, there is always a price increase for tomatoes during those months and a decrease until mid-August. Post-harvest losses due to tomato handling occur at various levels such as field or farm level, market level, transportation, storage and retailer level. In India, there is a 13% post-harvest loss on tomatoes, of which 3.04% is caused during transportation and storage.Since tomato is chilling sensitive, one of the major factors contributing to this loss is exposure of tomato fruit to low temperature in the cold chain. The recommended temperature range for storage and transportation of tomato is 10-13°C at 90% relative humidity, however, often the temperature is maintained at 3-6°C in most cold storage supply chain. Storage of tomato at the temperature < 10°C cold stress is induced, while at temperature below 4°C chilling injury is induced that leads to loss of aroma, appearance of blotch, softened texture, disease susceptibility and inability to adequate ripening. As tomato is a climacteric fruit, its ripening is induced and influenced by ethylene, which also plays significant role in cold stress management. This research work aims to study the effect of post-harvest cold stress on tomato and assess the role of ethylene in alleviation of cold-stress and modulation of the ripening process of tomato fruit under cold stress.

Keywords: Tomato production, post-harvest loss, cold stress, chilling injury, ethylene modulators

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